Nonneoplastic Changes in the Olfactory Epithelium—Experimental Studies

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Interest in the olfactory mucosa has increased in recent years, since it has been shown to possess a considerable amount of cytochrome P-450-dependent monooxygenase activity and a wide variety of chemicals have been identified as olfactory toxins. Many chemicals induce lesions of a general nature in the olfactory mucosa, i.e., inflammation, degeneration, regeneration, and proliferation, whereas others cause more specific effects.

Changes in the olfactory mucosa with reference to chemicals that initiate them are reviewed in this paper. Studies with 3-trifluoromethyl pyridine (3FMP) illustrate some of these general changes and show the importance of examining the olfactory mucosa at early time periods. The earliest damage seen by light microscopy was 6 hr after a single inhalation exposure to 3FMP, and by day 3, early regenerative changes were observed. Changes were seen by electron microscopy 30 min after an oral dose, and the primary site of toxicity appeared to be the Bowman's glands.

Although atrophy of nerve bundles in the lamina propria would be the expected consequence of severe necrosis of the sensory cells, this is not always the case. Exposure to irritants such as acetaldehyde, formaldehyde, and dimethylamine results in nerve bundle atrophy, but with chemicals such as 3FMP, 3-methylindole, and 3-methylfuran—which are activated by mixed-function oxidases—the nerve bundles remain intact. Future work, including metabolism studies, will provide more information on the mode of action of these chemicals.

Introduction

Twenty years ago the nitrosamines were recognized as an important class of chemicals inducing tumors in the posterior part of the nasal cavity. In 1964 Herrold (*1*,*2*) described olfactory neuroepithelial tumors in Syrian hamsters when diethylnitrosamine was administered by intragastric, intratracheal, or subcutaneous routes, and Pour et al. in 1974 (*3*) induced carcinomas in the olfactory epithelium when di-*n*-propylnitrosamine was administered subcutaneously, once weekly in a lifetime study.

However, the olfactory epithelium was rarely examined in short-term studies, and it is only within the last decade that this epithelium has been examined routinely in inhalation studies and become recognized as an important target site for toxicity. This was because of a variety of factors including the low incidence of spontaneous nasal cavity tumors in rodents, unfamiliarity of pathologists with normal nasal morphology, and technical difficulties encountered in the preparation of the nasal cavity for histological assessment.

One of the first descriptions of early nonneoplastic changes in the olfactory epithelium was in 1969 after the parenteral administration of diethylnitrosamine or dimethylnitrosamine to Syrian hamsters (*4*). The earliest detectable changes by light microscopy were spaces in the sensory layers because of the loss of cohesion between neighboring sensory cells, and within 48 hr there was olfactory epithelial necrosis. Another report of nonneoplastic changes in the olfactory epithelium was after subcutaneous administration of di-*n*-propylnitrosamine to Syrian hamsters when the epithelium was examined at 14 weeks (*3*). Focal atrophy of sensory cells, proliferating sustentacular cells intermingled with small cells and enlargement of cells of Bowman's glands were described. In animals that died later in the study, focal areas of squamous metaplasia and circumscribed hyperplasia in linear, papillary, and nodular patterns were observed.

Interest in this area increased in the 1980s when the olfactory mucosa was shown to possess a considerable amount of cytochrome P-450-dependent monooxygenase activity and when it was discovered that many chemicals were metabolized in this tissue (*5*–*9*). A wide variety of chemicals has now been shown to induce changes in the olfactory epithelium. Changes common to many of these chemicals, as well as more specific responses, will be described in this paper.

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General Pathological Changes in the Olfactory Epithelium

Changes in the olfactory epithelium may be induced when a chemical is administered by inhalation, oral, intratracheal, subcutaneous, intragastric, or intraperitoneal routes. There is a wide variety of chemicals reported in the literature that induce lesions in the olfactory epithelium, and some examples are tabulated in Tables 1 and 2 according to the route of administration.

Olfactory lesions may also be classified according to the mode of action of the chemical, i.e., direct or indirect. A direct-acting chemical is one where the parent compound is toxic to the olfactory epithelium, while a chemical acting indirectly is metabolized to a toxic intermediate, either in the olfactory epithelium or in a distant organ and then transported via the bloodstream to the olfactory epithelium where it has a toxic effect. Many of the direct-acting chemicals are gaseous irritants, such as chlorine (*17*) and acetaldehyde (*10*), and after exposure to these irritants, there is generally a distinct anterioposterior gradient of severity of damage.

Following exposure to these chemicals the most frequently affected site in the olfactory region of the nose is that lining the dorsal meatus. Furthermore, the respiratory epithelium is also affected. The majority of direct-acting nasal toxins affect this site in the nose, but metals such as zinc and nickel mainly show damage to the olfactory epithelium.

Conversely, chemicals that are metabolized to a toxic intermediate—that is, those with an indirect action—usually induce lesions in all or a large percentage of the olfactory epithelium, while the respiratory epithelium is only rarely affected. Examples of these indirect-acting chemicals are 3FMP (*35*) and 3-methylindole (*34*). Not all chemicals in Table 1 can be assumed to be direct acting. They were assigned to this table because the inhalation route was the only route of administration described in the literature. Methyl bromide (*40*), 3-methylfuran (*25*), and dimethylamine (*20*) are known to be metabolized.

Nonneoplastic changes in the olfactory epithelium can also be classified by the nature of the reaction. These changes are independent of whether the chemical has a direct or an indirect effect, and are more dose-related to the treatment regimen and the time of examination of the tissue after exposure. Olfactory changes fall into the following categories: inflammation, degeneration, regeneration/repair, and proliferation.

Table 1. Chemicals inducing olfactory lesions by inhalation.

Chemical	Reference
Acetaldehyde	(*10–12*)
Acrolein	(*13*)
Acrylic acid	(*14*)
Allyl glycidyl ether	(*15*)
Bis(chloromethyl)ether	(*16*)
Chlorine	(*17*)
1,2-Dibromo-3-chloropropane	(*18,19*)
1,2-Dibromoethane	(*18*)
Dimethylamine	(*20*)
Dimethylethylamine	(unpublished data)
Ethyl acrylate	(*21*)
Furfuraldehyde	(*22,23*)
3-Methylfuran	(*24,25*)
N-Methyl-formiminomethylester	(*26*)
Methyl bromide	(*27*)
Methyl isocyanate	(*28*)
Nickel subsulfide	(*29*)
Nickel sulfate	(*30*)
Vinyl chloride monomer	(*31*)
Zinc oxide	(unpublished data)

Table 2. Chemical inducing olfactory lesions by noninhalation routes.

Chemical	Reference
N' -Nitrosamines	(*3,4,32,33*)
3-Methylindole	(*34*)
3-Trifluoromethyl pyridine (3FMP)	(*35*)
Quanoxaline 1,4-dioxide	(*36*)
Procarbazine	(*37*)
p-Cresidine	(*38*)
Phenacetin	(*39*)

Inflammation

Inflammation may occur as a result of exposure to infectious organisms or as a response to toxic substances. Only the latter will be discussed. Exudate in the nasal cavity (Plate 1) composed of proteinaceous fluid, mucus, and inflammatory cells is a good indication that an inflammatory reaction is occurring. Cellular necrosis may occur, dependent on the severity of the inflammation, but whether the inflammation is a secondary response to necrosis, or is a primary event after exposure to toxic substances, remains unknown. In the olfactory region, inflammatory cells are more commonly seen in the lamina propria than in the epithelium itself, even though in the respiratory epithelium, there may be a marked intraepithelial infiltrate of inflammatory cells. Focal changes, such as erosion and ulceration may also occur. Inflammatory changes are nonspecific but are dependent on exposure dose or concentration and degree of necrosis and are seen more frequently after administration of irritants such as acetaldehyde (*11*) and chlorine (*17*). However, 3-methylfuran (*25*) and 3-methylindole (*34*)—both of which are activated by mixed-function oxidases—induce acute, severe serofibrinous, necrotizing rhinitis.

Degeneration

Degeneration resulting in atrophy or complete necrosis of the olfactory epithelium is a common change after administration of an olfactory toxin. One of the earliest changes described is increased intercellular space with subsequent progressive disruption of the epithelium because of loss of cohesion between neighboring cells. This change was seen after short-term exposure to

methyl bromide (*40*) and 48 hr after administration of diethylnitrosamine or dimethylnitrosamine (*4*). Further degenerative changes such as vacuolation, nuclear pyknosis, and frank necrosis of sensory cells results in an atrophic epithelium. when mice were exposed to dimethylamine (*20*) and rats to dimethylethylamine (unpublished data) there was complete loss of sensory cells, with only the basal and sustentacular cells remaining (Plates 2 and 3). After exposure to other chemicals including zinc oxide (unpublished data), 3-methylfuran (*25*), methyl bromide (*27*), diethylnitrosamine, dimethylnitrosamine (*4*), or 3FMP (unpublished data), complete desquamation of the olfactory epithelium occurs, with only basal cells remaining (Plate 4).

A logical consequence of the destruction of sensory cells is atrophy of the nerve bundles in the lamina propria (Plate 5) and, indeed, this is seen after treatment with acetaldehyde (*12*), formaldehyde (*41*), and dimethylamine (*20*). However, 3FMP (*35*), 3-methylfuran (*25*), and 3-methylindole (*34*) cause complete necrosis of the olfactory epithelium and, yet, the nerve bundles are preserved. These two groups of chemicals have a difficult mode of action, the latter three being activated by mixed function oxidases. As more chemicals that are activated by mixed function oxidases come to light, we hope to gain some insight into this phenomenon.

Regeneration/Repair

Neurogenesis in the olfactory epithelium is a dynamic process with a turnover time of approximately 30 days (*42*). Olfactory sensory cells are unique post-embryonic neurons in their ability to be replaced after injury (*43*), and the basal cell has now been established as the progenitor cell. Damage to the olfactory epithelium may completely resolve, or the epithelium may be replaced by either squamous or respiratory epithelium. The factors that control the type of differentiation during the recovery stages are as yet unknown. Frequently, damaged epithelium regenerates, but there is not complete resolution, as it does so in a disorganized fashion with atypical arrangements of the neural elements. Occasionally large bundles of axons are present within the epithelium (*42*) (Plate 6), and pseudoglandlike structures (*35*), possibly formed by infolding of the surface epithelium (Plate 7), may also be present. Early stages of regeneration must not be confused with squamous metaplasia that it superficially resembles. The regenerative process is described in more detail below where studies with 3FMP (*35*) are reported.

Olfactory epithelial respiratory metaplasia (Plate 8) has been reported after inhalation of acrylic acid (*14*), ethyl acrylate (*21*), dimethylamine (*20*), furfuraldehyde (*23*), and dimethylethylamine (unpublished data). It has not been confirmed whether this is a true respiratory epithelium or an epithelium that lost its sensory cells and was composed of basal and sustentacular cells. The surface of the epithelium resembles respiratory epithelium, but goblet cells are absent. According to Buckley et al. (*20*), the ciliated epithelial cells show intermediate characteristics of both sustentacular cells and columnar ciliated cells of normal respiratory epithelium. In my experience, respiratory metaplasia is more frequently seen in mice and may occur as a spontaneous change in old mice. After repeated exposure to acrolein (*13*), acetaldehyde (*11*), formaldehyde (*44*), or hexamethylphosphoramide (*45*), the olfactory epithelium was replaced by squamous epithelium. The squamous epithelium is presumably more resistant to the irritant effects of these gases. Squamous metaplasia of the olfactory epithelium is rarely seen in short-term studies, but it has been reported 5 days after a single IP injection of 3-methylindole (*34*).

Proliferation

Excessive proliferation of cells may result in epithelial hyperplasia or neoplasia. Pour et al. (*3*) reported hyperplasia changes in the olfactory epithelium when Syrian hamsters were exposed to di-*n*-propylnitrosamine. Hyperplasia preceded tumor development but was also seen simultaneously with tumors.

Examples of hyperplasia of all the olfactory cell types have been reported in the literature. After inhalation of ethyl acrylate (*21*), there was a decrease in the number of mature neurons in the olfactory mucosa with compensatory hyperplasia of basal cells. Rats exposed to dimethylamine showed basal cell hyperplasia in the olfactory epithelium (Plate 9), but this was not observed in mice (*20*), and atypical hyperplasia of basal cells of the olfactory epithelium was described when rats were exposed to 5000 ppm vinyl chloride for 52 weeks (*31*).

Hyperplasia of sensory cells was seen after rats were exposed to 3FMP for 13 weeks (Plate 10) (*35*), and proliferating sustentacular cells have been described after treatment with di-*n*-propylnitrosamine (*3*).

Unusual Nonneoplastic Lesions in the Olfactory Epithelium

Cystlike Structures in the Lamina Propria

Cysts (Plate 11) in the lamina propria were described in Syrian hamsters after exposure to furfuraldehyde vapor (115–552 ppm) for 13 or 52 weeks (6 or 7 hr/day, 5 days/weeks) (*22,23*). Homogeneous, eosinophilic, periodic-acid, Schiff positive material, cellular debris, and polymorphonuclear leucocytes often filled the lumena of these cysts that were lined by flat, cuboidal, or tall columnar cells with cilia or ciliumlike projections. Occasionally the epithelium of the cystlike structures was seen to be continuous with altered surface epithelium, suggesting that infolding or downward growth of surface epithelium are major factors in the genesis of these cystlike structures. However, genesis from Bowman's glands is a possible alternative.

Cytomegaly of Bowman's Glands

Cytomegaly (Plate 12) was another unusual lesion seen when hamsters were exposed to furfuraldehyde (*22,23*). The epithelial cells of the glands of Bowman were very large with abundant cytoplasm and huge nuclei. The lesion has not been reported after treatment with other compounds.

Sensory Cells in the Lamina Propria

Sensory cells (Plate 13) have been described in rats 35, 70, and 157 days after a single 50-ppm exposure of 3FMP (unpublished data) and when rats were exposed to 10 ppm 3FMP 6 hr/day, 5 days/week for 13 weeks (*35*). It was also seen when hamsters were exposed to furfuraldehyde (*22,23*). There were nests of sensory cells, with their characteristically prominent nucleoli, lying in the lamina propria as irregular or well-circumscribed groups invariably resting on a basal lamina. Downward growth of surface epithelium seems to be the most plausible explanation of the occurrence of sensory cells in the lamina propria. Neither recovery nor progression of this lesion was seen after a withdrawal period of six months (*23*).

Sustentacular Cell Karyomegaly

Some nuclei of sustentacular cells (Plate 14) were observed to be approximately two to three times normal size when rats were exposed to 50 ppm 3FMP for 13 weeks (*35*). This is a personal observation and there does not appear to be any other record of this lesion in the literature.

Effects of 3-Trifluoromethyl Pyridine in Rats

3FMP is a by-product in the manufacture of 2-chloro-5-trifluoromethyl pyridine, which is an intermediate in the manufacture of a herbicide. It is metabolically activated by mixed-function oxidases to a toxic intermediate in the olfactory mucosa. Lesions occurred in the olfactory mucosa after inhalation exposure, oral dosing, or IP injection, and autoradiographic studies indicated the presence of the compound in the olfactory region of the nasal mucosa 30 min after oral dosing.

Single Exposure Study

Rats were exposed to 0.1, 1.0, 10, or 50 ppm 3FMP for 6 hr and necropsied at 6 and 24 hr and on days 3, (48 hr after exposure) 5, 8, 11, 35, 70, and 157 (unpublished data).

The earliest change seen by light microscopy was at 6 hr when the olfactory epithelium had an undulating appearance apparently because of focal atrophy of sensory cells. There was a reduction in the number of layers of sensory cells from 8 to 3 (Plate 15), unlike the effect seen after inhalation of methyl bromide (*14*), where there was folding of the epithelium. In other areas there was focal epithelial necrosis. Severe diffuse necrosis and desquamation of the olfactory epithelium occurred after 24 hr with only basal cells remaining in some areas (Plate 4). By day 3, there were signs of early regeneration with desquamated epithelial cells overlying 1 to 2 layers of basophilic, regenerating cells (Plate 16). Further regeneration had occurred by day 11, but the entire olfactory epithelium had a disorganized appearance with vacuolated cells, glandlike structures, and a reduction in the apical cytoplasm. Olfactory cilia and microvilli of sustentacular cells appeared to be absent from the surface of the epithelium (Plate 17). By days 35 and 70, there were only focal areas of disorganization, vacuolation, and gland formation. Axon bundles were observed in the epithelium and sensory cells were present in the lamina propria. The epithelium had virtually regenerated by day 157, but occasionally axon bundles were observed in the epithelium and sensory cells were present in the lamina propria. Nerve bundles remained intact at all time periods.

10-Day Subacute Study

Rats were exposed six hr/day for 10 consecutive days to 0, 17, 83, or 329 ppm 3FMP (*35*). Nasal passages were examined on day 11. The lesion in the olfactory epithelium was of the same severity at all exposure levels and identical with that seen 10 days after a single exposure.

The most severe damage occurred 24 to 48 hr after a single exposure to 3FMP. After multiple exposures there was less damage to the olfactory cells and evidence of regeneration. This indicates that the 3FMP-exposed olfactory mucosa has probably adapted (possibly biochemically) and the cells have become more resistant to 3FMP.

The work with 3FMP has provided some insight into the process of regeneration of damaged olfactory epithelium. Regeneration occurs at a very early stage and it is important to examine the nasal cavity at short-time intervals after exposure in order to assess the damage.

Electron Microscopy

The initial effect in rats dosed orally with 3FMP (50 mg/kg) (unpublished data) was a dilatation of smooth endoplasmic reticulum in the cells of Bowman's glands. This was first apparent after 30 min. The effect was more prominent in those cells nearest to the excretory duct of the gland. If the duct of the Bowman's gland is the primary route of excretion, this would explain the undulating effect in the olfactory epithelium as cells nearest to the glandular duct in the epithelium would be most severely affected. That the Bowman's gland was the first site affected indicates that the circulatory route was of primary importance.

Summary

In recent years interest in the olfactory mucosa as a primary site for toxicity has increased. A wide variety of chemicals induce changes in this region of the nasal passages. General effects in this tissue include inflam-

mation, degeneration, regeneration/repair, and proliferation, which are seen after administration of chemicals by inhalation or by other routes. When 3FMP was administered to rats by the inhalation route, the earliest damage seen by light microscopy was at 6 hr and by electron microscopy, at 30 min. The most severe effects were seen at 24 hr, and early regeneration had occurred by day 3, indicating the importance of examination of the olfactory epithelium at early time periods. The primary site for toxicity appeared to be the epithelial cells of Bowman's glands.

Atrophy of nerve bundles in the lamina propria may or may not result after necrosis of sensory cells. With irritants such as acetaldehyde, formaldehyde, and dimethylamine there is atrophy of nerve bundles; with chemicals such as 3FMP, 3-methylindole, and 3-methylfuran, which are activated by mixed-function oxidases, the nerve bundles remain intact.

In the future, many other chemicals may be shown to cause changes in this region of the nasal passages, and further work including metabolism studies will provide more information on the mode of action of these olfactory toxins.

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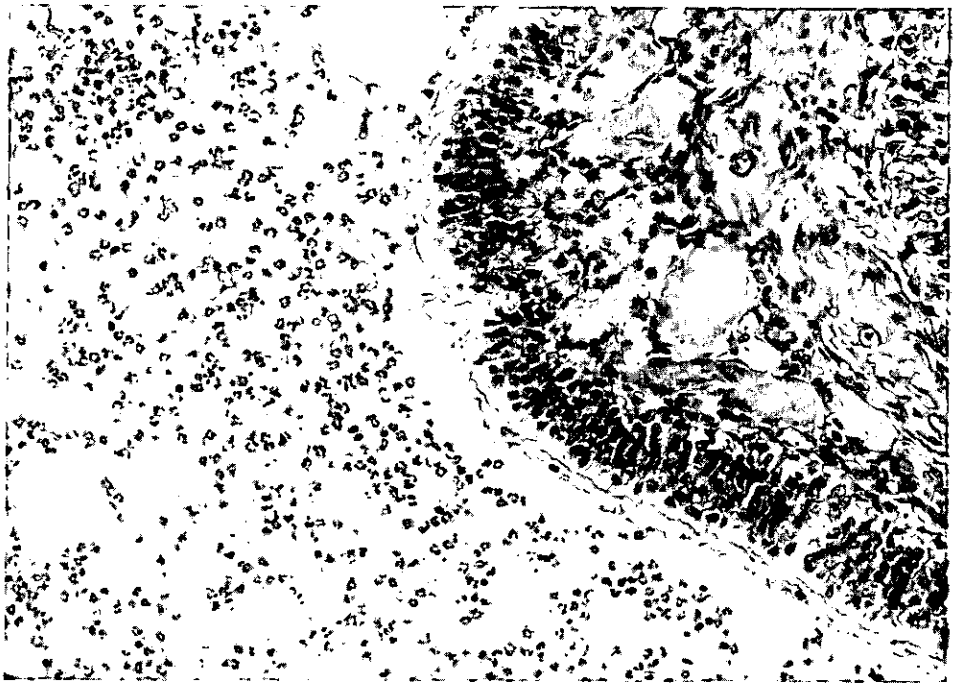

PLATE 1. Exudate composed of mucus, proteinaceous fluid, and polymorphonuclear leukocytes in the ethmoid region of the nasal cavity of a rat exposed to an irritant fume for 6 hr. H&E, ×360.

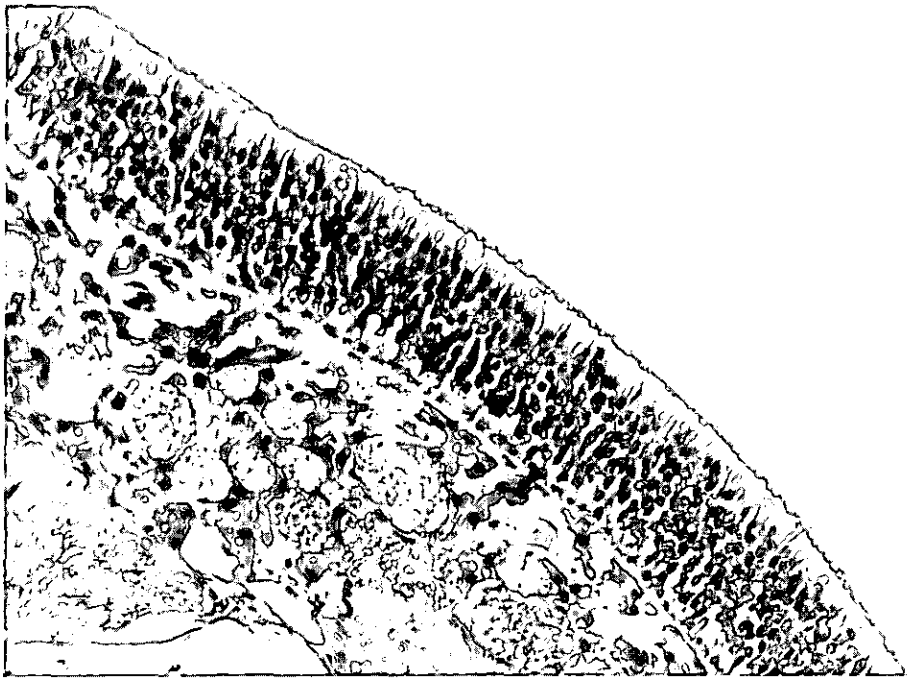

PLATE 2. Normal olfactory epithelium of an untreated rat. H&E, ×360.

PLATE 3. Olfactory epithelium in the dorsal meatus of a rat exposed to 300 ppm dimethylethylamine for 10 days. Loss of sensory cells with only basal and sustentacular cells remaining. H&E, ×360.

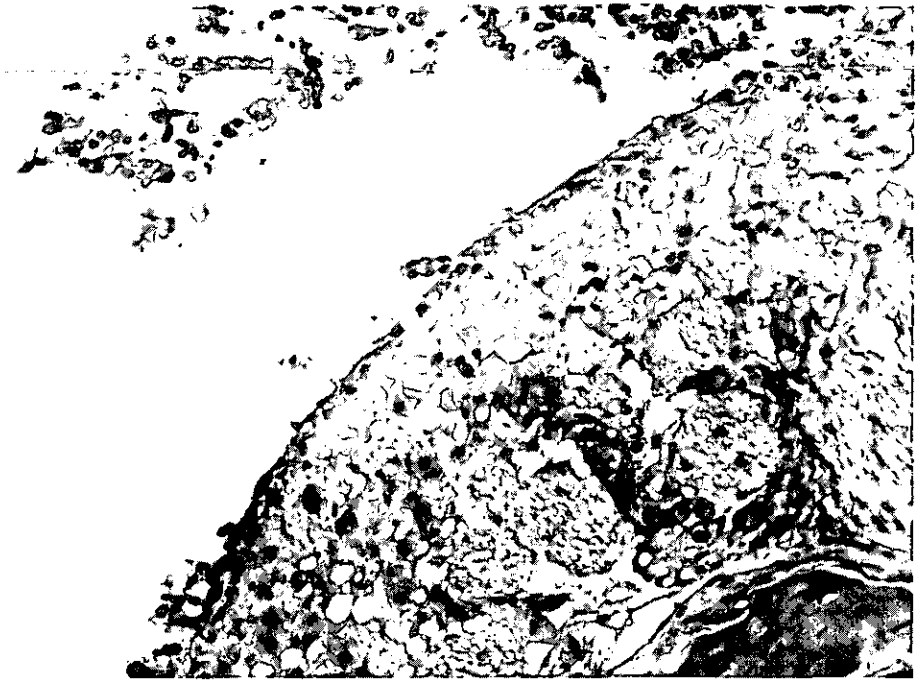

PLATE 4. Desquamation of the olfactory epithelium of a rat 48 hr after a single 6-hr exposure to 50 ppm 3-trifluoromethyl pyridine. Only basal cells remaining. H&E ×630.

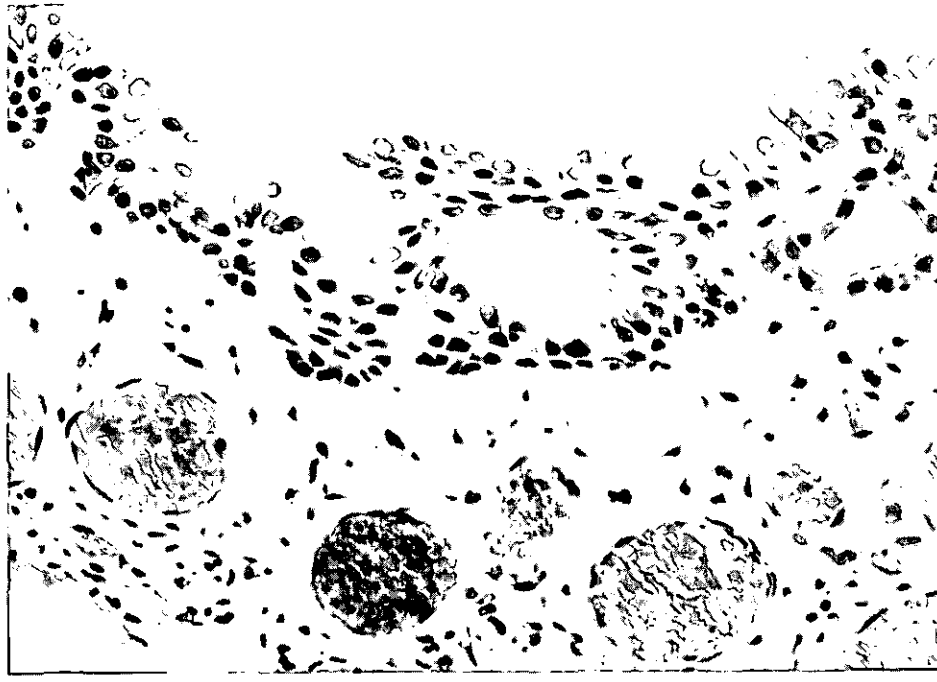

PLATE 5. Loss of nerve bundles from the lamina propria in the olfactory region of a rat exposed to 175 ppm dimethylamine for 6 months. H&E, ×360.

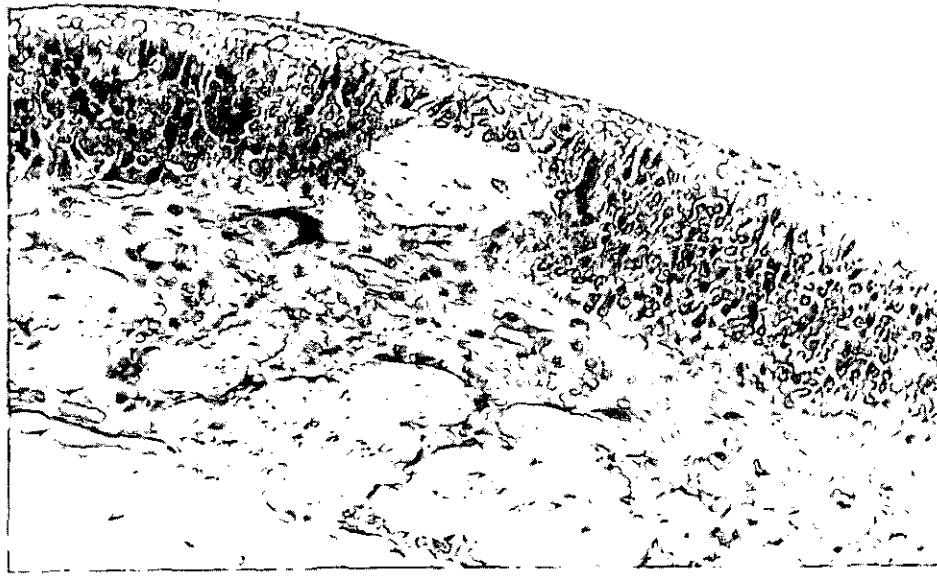

PLATE 6. Axon bundles within the regenerating olfactory epithelium of a rat 70 days after a single 6-hr exposure to 50 ppm 3-trifluoromethyl pyridine. H&E, ×360.

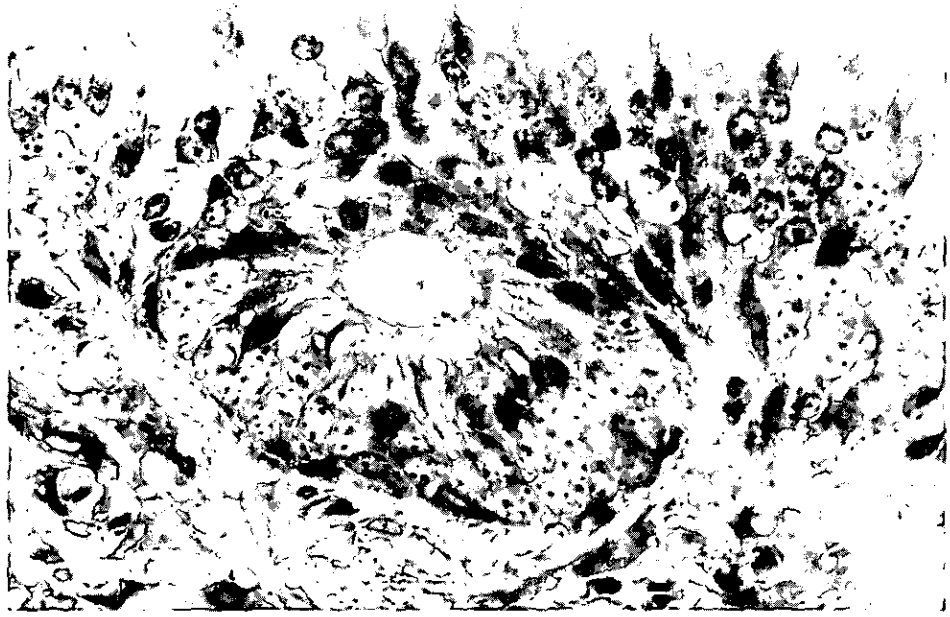

PLATE 7. Glandlike structure within the regenerating olfactory epithelium of a rat 10 days after a single 6-hr exposure to 50 ppm 3-trifluoromethyl pyridine. H&E, ×360.

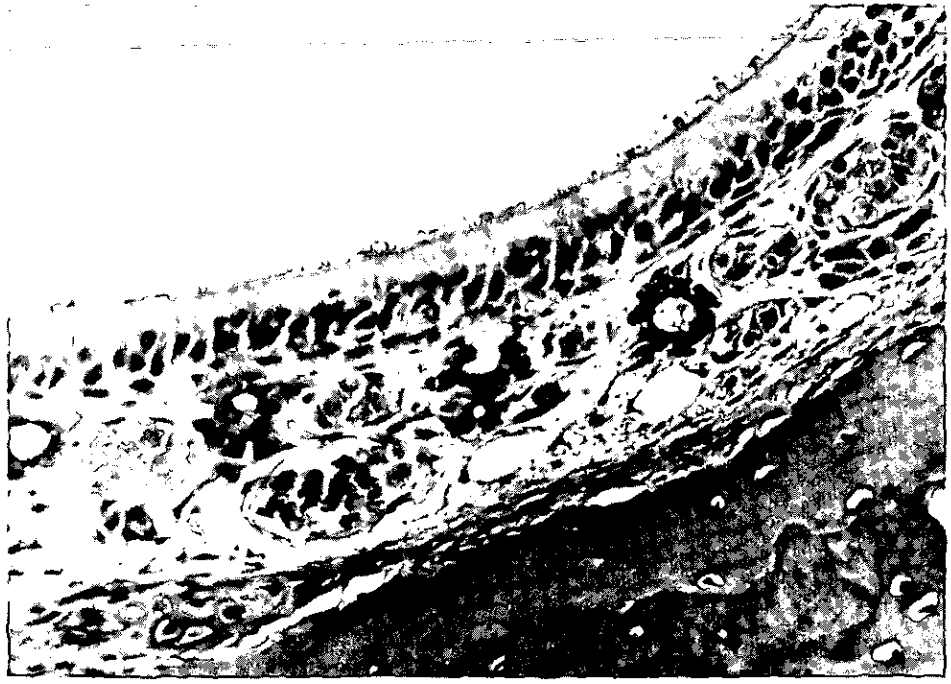

PLATE 8. The *dorsal meatus* of a rat exposed to 300 ppm dimethylethylamine for 10 days followed by a 14-day recovery period. The olfactory epithelium in this region of the dorsal meatus has been replaced by a ciliated respiratorylike epithelium. H&E, ×360.

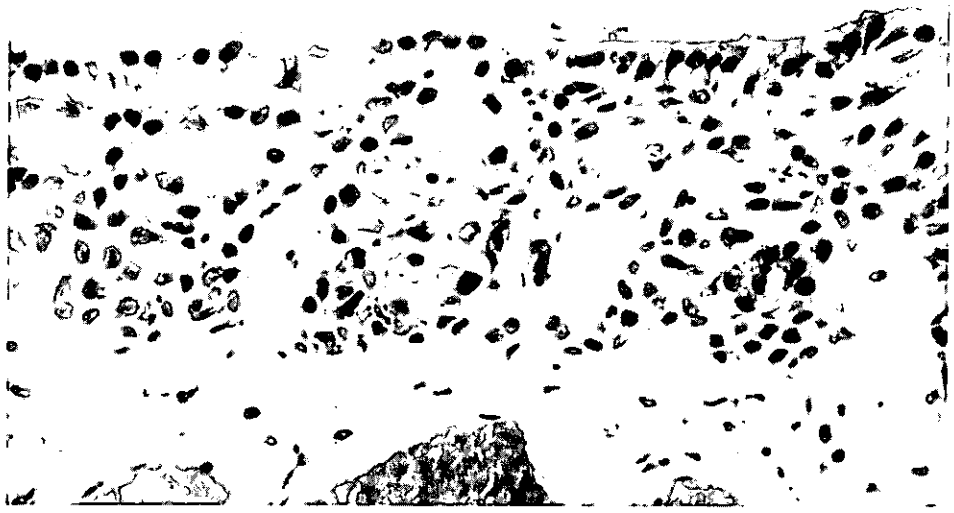

PLATE 9. Basal cell hyperplasia in the olfactory epithelium in the dorsal meatus of a rat exposed to 175 ppm dimethylamine for 6 months. H&E, ×360.

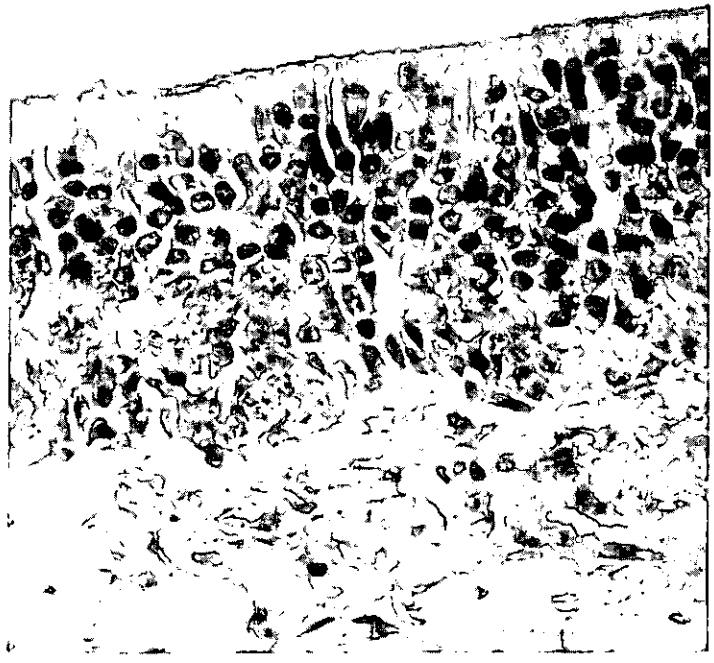

PLATE 10. Hyperplasia of sensory cells in the olfactory epithelium after rats were exposed to 10 ppm 3-trifluoromethyl pyridine for 13 weeks. H&E, ×630.

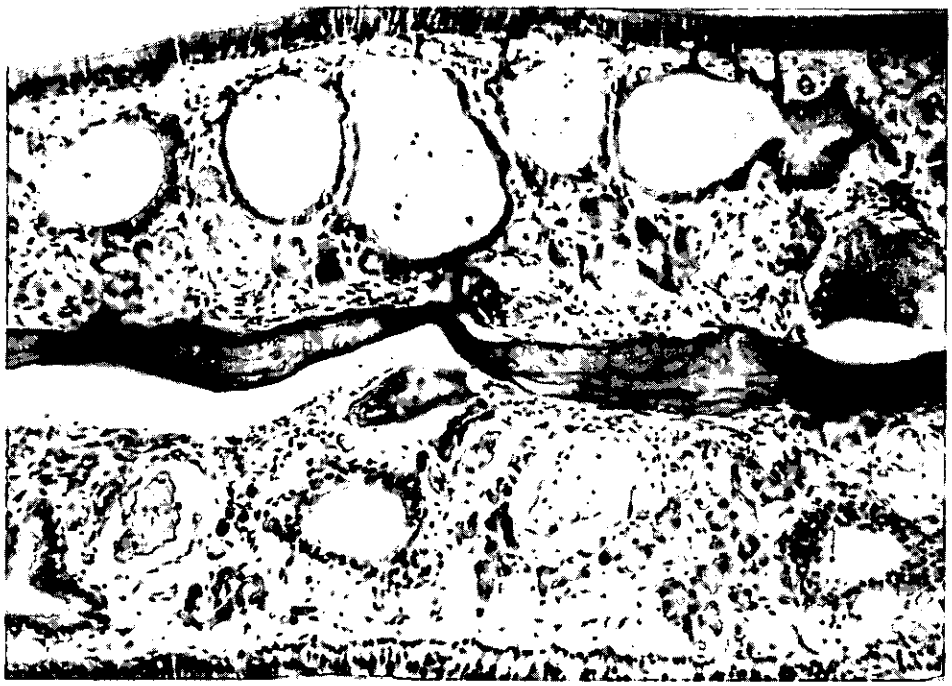

PLATE 11. Cystlike structures in the lamina propria in the olfactory region of a Syrian hamster exposed to 552 ppm furfuraldehyde for 13 weeks. H&E, ×360.

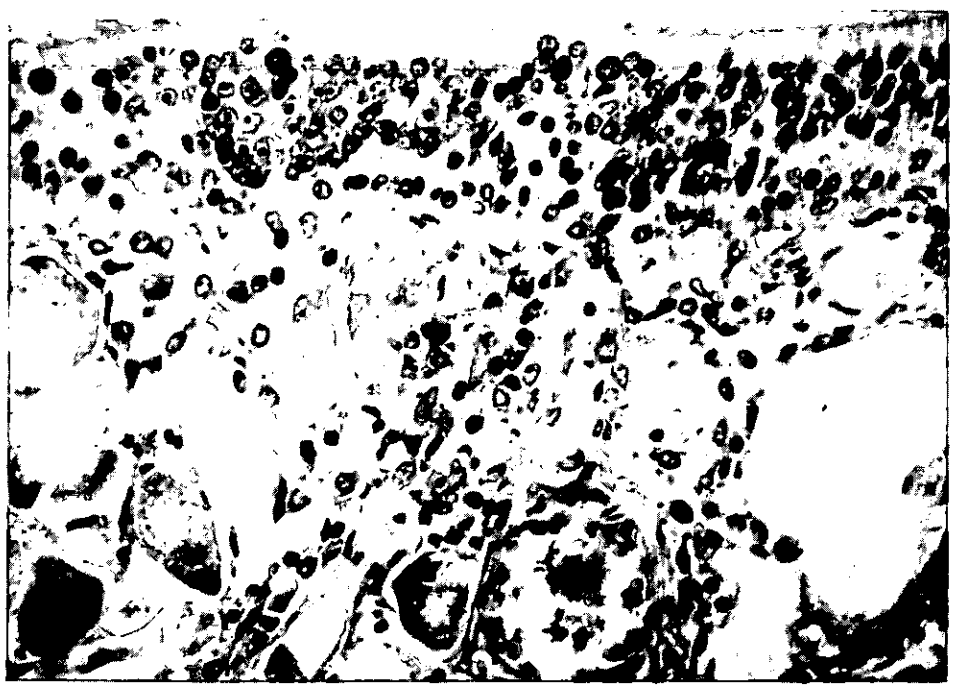

PLATE 12. Enlarged epithelial cells of Bowman's glands in a Syrian hamster exposed for 52 weeks to 400 ppm furfuraldehyde (decreased to 250 ppm after 45 weeks) followed by a recovery period of 29 weeks. H&E, ×360.

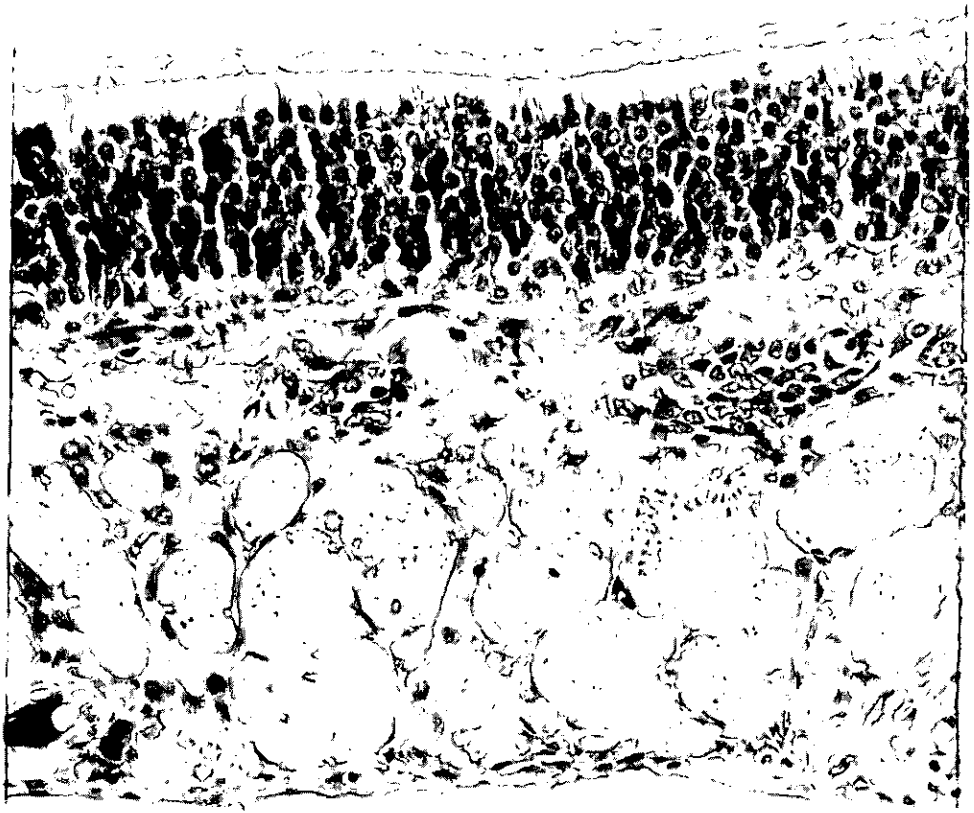

PLATE 13. Sensory cells in the lamina propria in the olfactory region of a rat exposed to 10 ppm 3-trifluoromethyl pyridine fo
weeks. H&E, ×360.

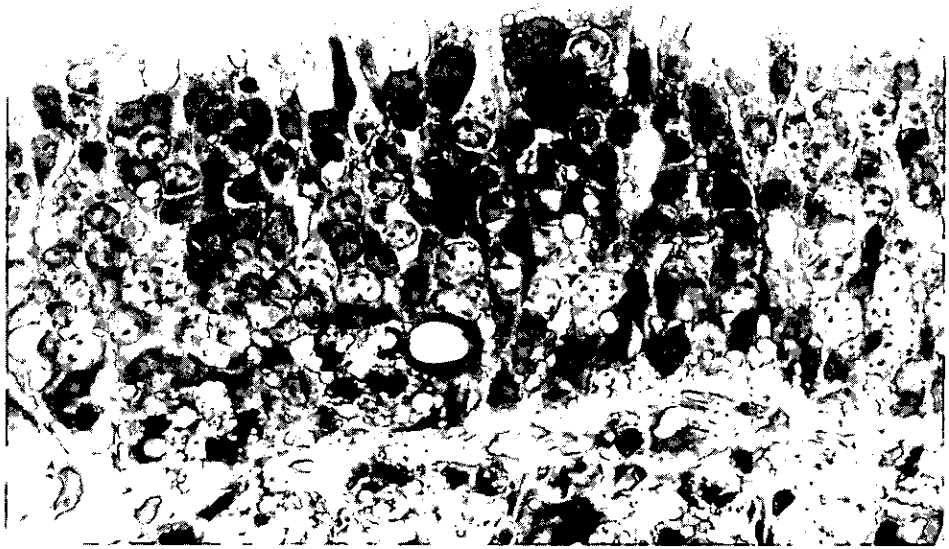

PLATE 14. Sustentacular cell karyomegaly in a rat exposed to 10 ppm 3-trifluoromethyl pyridine for 13 weeks. H&E, ×900.

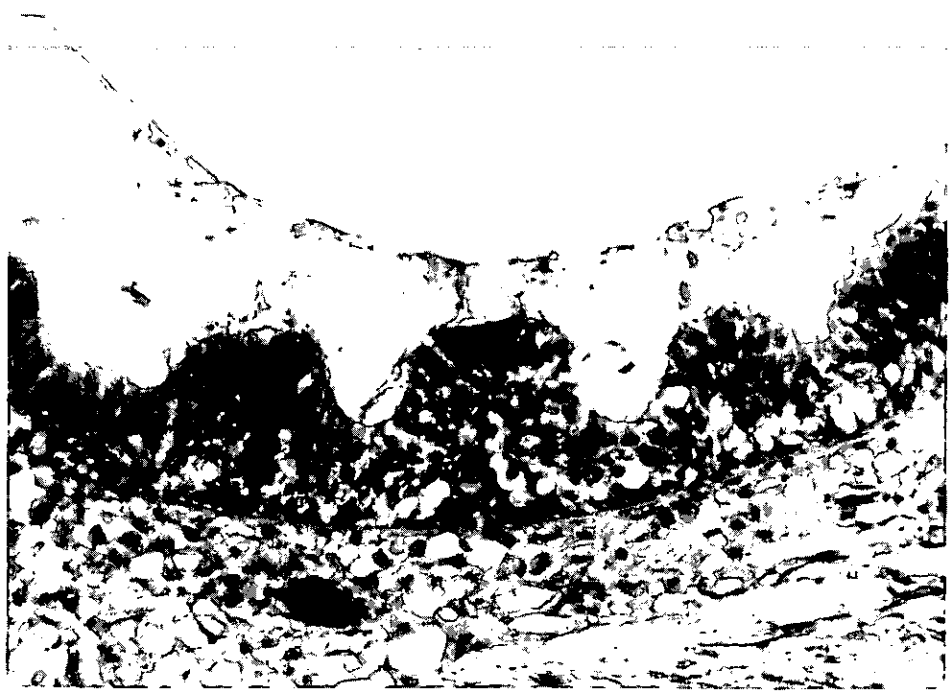

PLATE 15. Rat olfactory epithelium with an undulating appearance due to focal reduction in number of cell layers immediately after a single 6-hr exposure to 50 ppm 3-trifluoromethyl pyridine. H&E, ×360.

PLATE 16. Undifferentiated regenerating epithelium underlying desquamated olfactory epithelium in a rat, 48 hrs after a single 6-hr exposure to 50 ppm 3-trifluoromethyl pyridine. H&E, ×630.

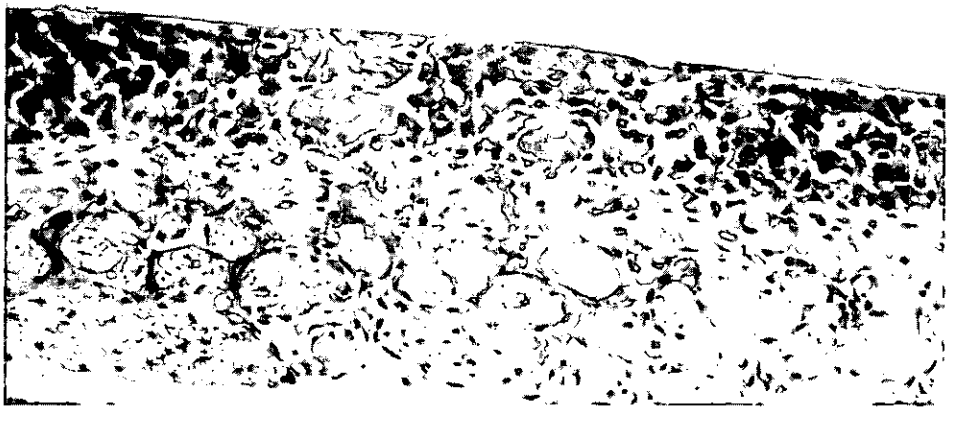

PLATE 17. Disorganized, regenerating olfactory epithelium in a rat 10 days after a single exposure to 50 ppm 3-trifluoromethyl pyridine. H&E, ×360.